Supplemental Material

Supplementary Fig. 1: T_{conv} cell contact potentiates T_{reg} suppression.

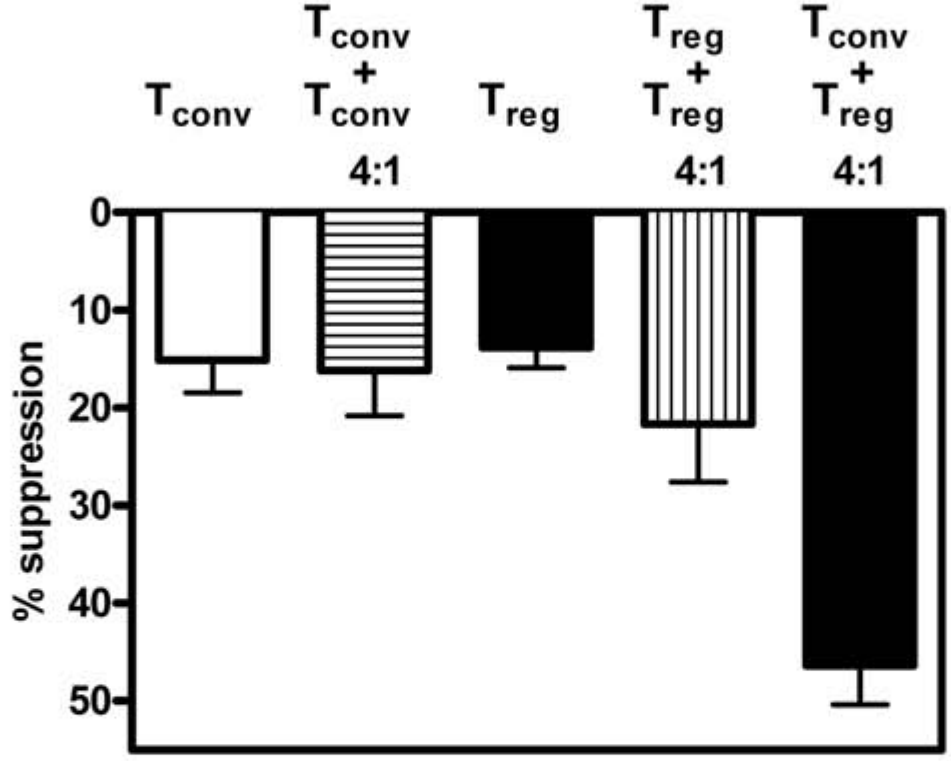
Supplementary Fig. 2: Purify of and Foxp3 expression in sorted T_{conv} and T_{reg}.

Supplementary Fig. 3: IL-2 secretion in responder T_{conv}

Supplementary Fig. 4: Capacity of polarized effector T cells to mediate suppression

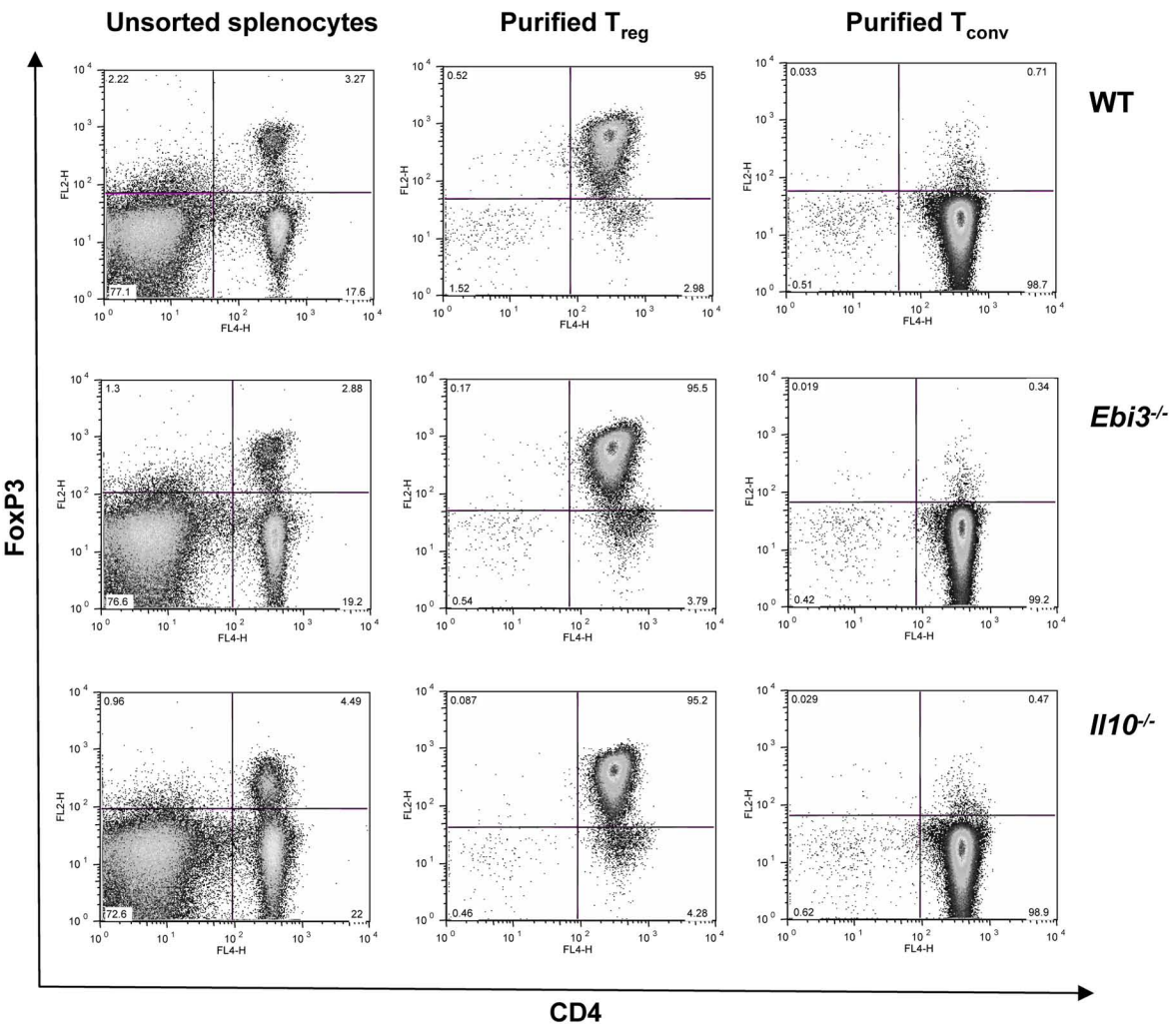
Supplementary Table I: Real-time PCR primer and probe sequences

Supplementary Fig. 1



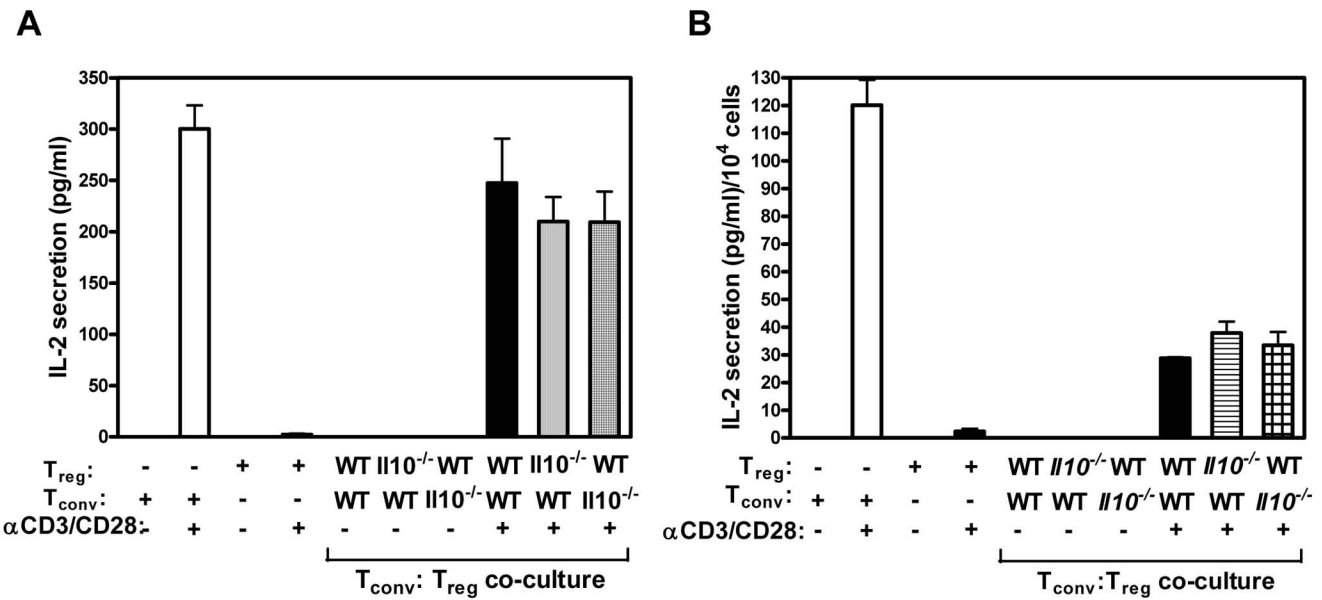
T_{conv} cell contact potentiates T_{reg} suppression. T_{conv} or T_{reg} cells from C57BL/6, mice were purified by FACS. Cells assayed for regulatory capacity (T_{conv} or T_{reg} alone or in combination at a 4:1 ratio) were cultured in the top chambers of a Transwell™ culture plate as indicated. Freshly purified wild-type "responder" T_{conv} were cultured in the bottom chamber of the 96-well flat bottom plates in medium containing anti-CD3/anti-CD28-coated latex beads. After 60 h in culture, top chambers were removed and [^{3}H]-thymidine was added directly to the responder T_{conv} cells in the bottom chambers of the original Transwell™ plate for the final 8 h of the 72 h assay. Cultures were harvested and cpm determined.

Supplementary Fig. 2



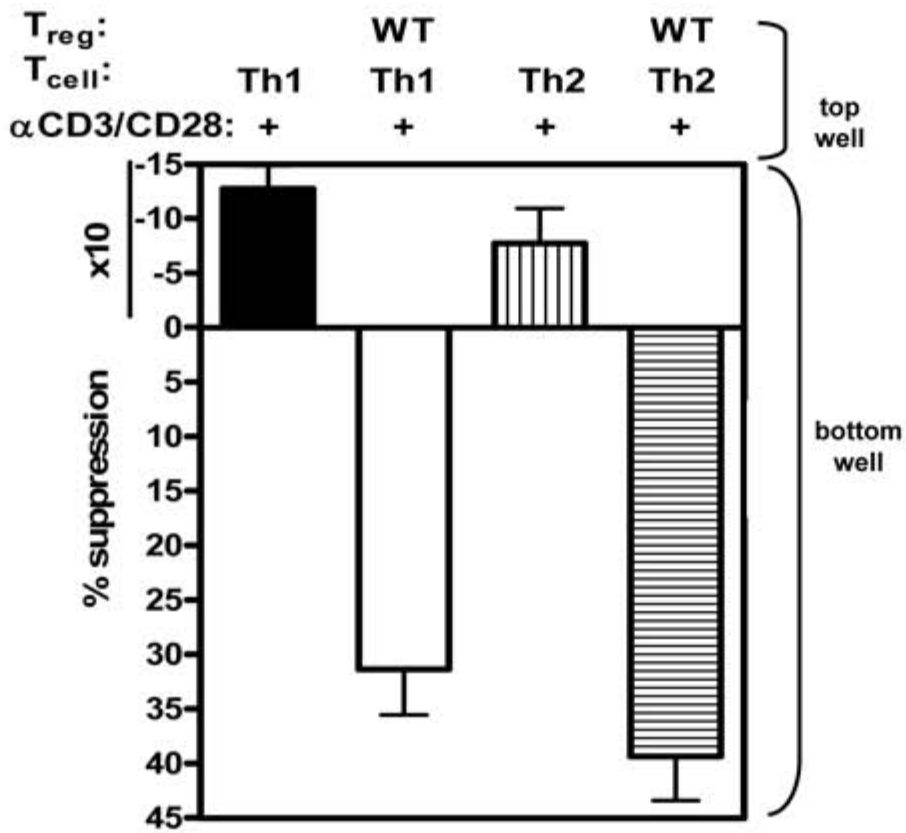
Purify of and Foxp3 expression in sorted T_{conv} and T_{reg}. WT, *Il10*$^{-/-}$, *Ebi3*$^{-/-}$ splenocytes were processed and red blood cells lysed prior to staining with anti-CD4 and anti-Foxp3 antibodies. In parallel, T_{conv} (CD4+CD25-CD45RB^{hi}) and T_{reg} (CD4+CD25+CD45RB^{lo}) were sorted and purified cells were subsequently stained with anti-CD4 and anti-Foxp3 antibodies and % Foxp3+ cells were determined by FACS.

Supplementary Fig. 3



IL-2 secretion in responder T_{conv}. Supernatants were collected from purified T_{conv} or T_{reg} cells cultured in the presence or absence of αCD3/CD28 stimulation or T_{conv}: T_{reg} co-cultures under indicated conditions. IL-2 secretion was measured using Luminex™ technology. (A) Total IL-2 secretion in cell culture supernatant (B) IL-2 secretion relative to the number of T_{conv} cells present in the culture (top and bottom chambers of the Transwell™. Data represent the mean ± SEM of 3 independent experiments.

Supplementary Fig. 4



Capacity of polarized effector T cells to mediate suppression. MACS purified T cells were cultured under polarizing conditions for 6 d (Th1: rIL-12, αIL-4, Th2: rIL-4, αIFNγ). On day 6, cells were collected, washed and used in functional assays. Th1 or Th2 cells were cultured in combination with Treg at a 4:1 ratio in top chambers of a Transwell™ culture plate as indicated. Freshly purified wild-type "responder" T_{conv} were activated in the bottom chambers and their proliferation was measured by [3H]-thymidine incorporation. Data represent the mean ± SEM of 3-5 independent experiments. Statistical analysis: *$p< 0.05$, ** $p< 0.01$. Counts per minute of T_{conv} cells activated alone were 25,000 – 60,000.

Supplementary Table 1

	Forward	Reverse	Probe
Ebi3	AGCAGCAGCCTCCTAGCCT	ACGCCTTCCGGAGGGTC	ACGCCTTCCGGAGGGTC
Il12 a (p35)	TGGCTACTAGAGAGACTTCTTCCACAA	GCACAGGGTCATCATCAAAGAC	AAGAGGGAGCTGCCTGCCCCC
Il10	TAGAGCTGCGGACTGCCTTC	AGTCGGTTAGCAGTATGTTGTCCAG	ACTTTCTTTCAAACAAAGGACCA
Il2	CCTGAGCAGGATGGAGAATTACA	TCCAGAACATGCCGCAGAG	CCCAAGCAGGCCACAGAATTGAAAG
ß a ctin	ACCCACACTGTGCCCATCTAC	AGCCAAGTCCAGACGCAGG	AGGGCTATGCTCTCCCTCACGCCA

Real-time PCR primer and probe sequences.